Spatial distribution of larvae of the mosquito *Culex tritaeniorhynchus summorosus* in a rice field area*

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Abstract

Spatial distribution of *Culex tritaeniorhynchus summorosus* larvae was studied in a rice field area surrounded by low hills, common in Nagasaki Prefecture. Rice fields in the area were about 15 hectares (roughly 500 rice fields), and among them 100 rice fields made of 11 groups were selected as study plots from various parts of the area. The study was done from May to October and the results were as follows. Larvae of *Culex tritaeniorhynchus summorosus* tended to concentrate in rice fields situated in some part of the whole area at a certain time and the position of such part changed during the period of mosquito occurence. Moreover the density level differed, sometimes markedly, among rice fields situated closely in a same group. These results are interesting in relation to the population study of this mosquito. Firstly, they ascertain our previous report that the number of rice fields examined should be increased, even though the number of dips taken from a rice field is decrease to only one, for estimating the relative density of the whole area in maximum efficiency. Secondly, they show that one rice field should be considered as one of natural unit for larval population in a rice field area. The latter seems to be very significant for understanding the processes of population fluctuation of this species, for density effects by overcrowding of larvae may act in some rice fields, while density in many other rice fields is remained low.

Introduction

The number of the mosquito *Culex tritaeniorhynchus summorosus*, the principal vector of Japanese encephalitis in Japan, fluctuates greatly both seasonally and annually. Understanding of the processes causing such fluctuations is considered

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essential for attempting the integrated control of this mosquito.

This species breeds mainly in rice fields, at least, in Japan, therefore seasonal prevalences of larvae and factors affecting them in rice fields have been reported from various regions of this country (Ori *et al.*, 1963; Ogata & Nakayama, 1963; Ishino *et al.*, 1966; Buei *et al.*, 1968; Makiya, 1970, 1971 and Nakamura *et al.*, 1971), showing that the survival rate of larvae in rice fields is affected by many factors such as water management, insecticides, food supply and so on. Their results show also that both the density level and the pattern of seasonal prevalence differ, sometimes very markedly, among rice fields lying in the same rice field area. For explaining the processes producing such differences, it may be useful not only to examine the condition of each rice field, but also to observe the distribution of larvae in a whole area containing the rice fields in question. However, the distribution of *Culex tritaeniorhynchus summorosus* larvae in a rice field area is little known at present. It was reported that in a sloping area the larvae of this species were found mainly in lower rice fields, but spread to upper ones at the peak of population fluctuation in late July (Yoshitake Wada & Watanabe, 1971). This may be one feature of the larval distribution of this species. Also they suggested that water temperature of rice fields might have had some influences on larval distribution. In this paper, seasonal change in the distribution of *Culex tritaeniorhynchus summorosus* larvae in a rice field area surrounded by low hills, common for Nagasaki Prefecture, will be described and also the processes causing such change will be discussed.

Before going further, we wish to express our sincere appreciation to Dr. N. Omori, the former professor of our Department for his constant encouragement throughout the course of the study. Our thanks are also due to Messrs. M. Yogata, M. Ueda and K. Kurokawa for their helpful assistance in the field work.

Place and method

The study was done in a rice field area of Mogi, near Nagasaki City. This area is situated in a small basin surrounded by low hills, and rice fields in this area are about 15 hectares (roughly 500 rice fields), as is shown in Fig. 1. Several pigsties were found around rice fields and 10 to 20 pigs were reared in each pigsty. As *Culex tritaeniorhynchus summorosus* is strongly zoophilic (Wada *et al.*, 1967, 1970), and as no other large mammals were reared in this area, pigs were certainly main blood source. The censuses were done from May to October, 1970 and the progress of rice plant cultivation during the course is shown in Table 1. This area is a single crop area and rice fields are left as fallow fields after a harvest in late October. Ploughing and making nursery bed were started in early and middle May, respectively in 1970. Transplantation of the rice plant was done at the beginning of July and since then most rice fields had water more or less

Table 1. The number of rice fields falling in each stage of rice plant cultivation.

No.	Date	The stage of rice plant cultivation				Total
		Fallow field	Ploughed field	Nursery bed	Rice field	
1	May 8	95 (25)	5			100 (25)
2	May 13	89 (31)	11			100 (31)
3	May 19	77 (43)	20 (3)	3		100 (46)
4	May 25	57 (12)	30 (3)	13		100 (15)
5	Jun. 2	43 (19)	37 (1)	20		100 (20)
6	Jun. 9	43 (26)	36 (10)	21		100 (36)
7	Jun. 16	42 (11)	37	21		100 (11)
8	Jul. 2	19 (3)	37 (3)	16 (4)	28	100 (10)
9	Jul. 9	7 (4)			93	100 (4)
10	Jul. 22	7 (5)			93	100 (5)
11	Jul. 29	7 (6)			93 (1)	100 (7)
12	Aug. 6	7 (7)			93	100 (7)
13	Aug. 11	7 (7)			93	100 (7)
14	Aug. 20	7 (6)			93 (3)	100 (9)
15	Sep. 1	7 (5)			93	100 (5)
16	Sep. 16	7 (6)			93	100 (6)
17	Oct. 9	7 (7)			93 (27)	100 (34)

In parenthesis the number of rice fields with no water is shown.

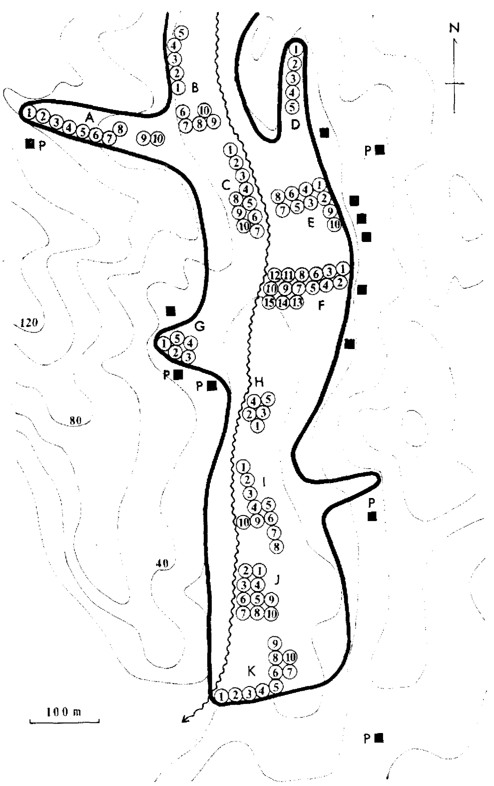


Fig. 1. A map of study area, rice fields being developed within a thick line. The rice fields examined are shown by white circles. The capitals from A to K and the figures in circles indicate the names of rice field groups and the rice field numbers in each group. P : pigsty, ■ : house.

till October, excepting several ones which were left to be fallow fields throughout the year. Not a small number of rice fields were filled with water even before transplantation. Also in some other rice fields, there was water in footprints after a rain before transplantation.

From various parts of the area, 100 rice fields made of 11 groups (A to K), each containing 5, 10 or 15 rice fields, were selected and fixed as study plots throughout the year. Three dips were taken from each rice field if it had any water,

Table 2. Density levels for sequential sampling of *Culex tritaeniorhynchus summorosus* larvae in a rice field (from Wada, Mogi & Nishigaki, 1971a).

Density level	Mean number of larvae per dip
Low	– 0.23
Moderate	0.23 – 5.09
High	5.09 –

Table 3. Sequential sampling table for *Culex tritaeniorhynchus summorosus* larvae in a rice field, when α and β are both 0.4 (from Wada, Mogi & Nishigaki, 1971a).

No. of dips	Cumulative number of larvae		
	Low	Moderate	High
1		1	10 ～
2	0	1 ～ 6	15 ～
3	0	2 ～ 11	20 ～

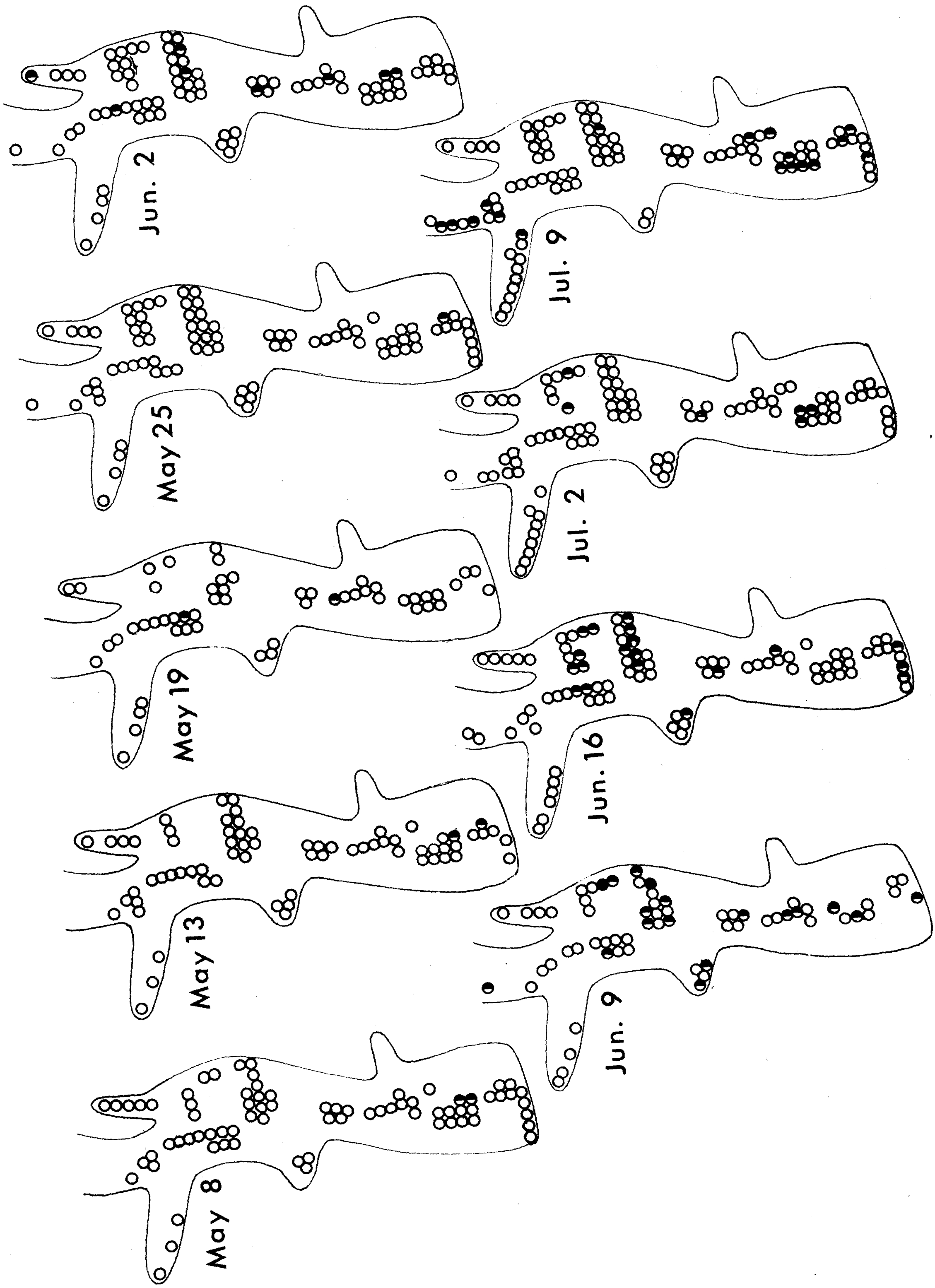
and the contents of each dip were concentrated using a tool similar to a plankton net into a small bottle with some formalin for later counting of larvae (pupae inclusive). Based on the numbers of larvae by the successive 3 dips, the density level (Table 2) of each rice field was determined by sequential sampling table in which α and β were both 0.4 (Table 3). In this method the density level of each rice field can be determined, for example, as moderate, when cumulative number of larvae dipped in a rice field falls, for the first time, into moder ate range of the sequential sampling table. When the cumulative number of larvae did not fall into any of the three density levels by 3 dips and remained to be intermediate between moderate and high levels, it was designated as moderate-high. (Low-moderate case does not occur if the sequential sampling table of Table 3 is used.) Here α and β are the probabilities of erroneous determination at some particular mean densities (critical densities) and their value 0.4 means rather low precision level (as for critical densities see Wada *et al.*, 1971a). However, the probabilities of erroneous determination vary with the mean density, therefore the actual rate of erroneous determination hwen applied to the field data depends also on the frequency distribution of mean densities in field. According to Wada *et al.* (1971a), when the number of rice fields falling into respective density levels by this method (i.e., the sequential sampling method in which α, β is 0.4 and the number of dips in each rice field is limited to 3) was compared with that by averaging the number of larvae in 10 dips, the rate of different determination was about 10% of total 221 rice fields censused in this area from July to October, 1968. For further explanation, see that paper.

Results obtained

Density levels of rice fields examined on each day are shown in Fig. 2. In this figure rice fields with no water are not shown. It is clearly seen from the figure that density level of larvae differed, sometimes markedly, among rice fields situated closely in a same group. Nevertheless rice fields falling into moderate or high density level tended to concentrate in some parts of the whole rice field area and the position of those parts changed during the year, although significance of departure from random distribution can not be tested because of too small number of groups for such statistical test. In May, larvae were found mainly in the lower (southern) part. In June they became to be spread over the whole area, but were found concentrated in the middle part, i.e., group F on June 9 and both E and F on June 16. Density decreased temporalily on July 2 perhaps, at least partially, owing to heavy rainfall in late June, but recovered soon and larvae spread over the whole area. On July 9 many larvae were found in group J in the lower part and B for the first time in the upper part. On July 22 density increased continuously and many larvae were collected in upper part such as group A and D where density had remained at low level almost completely till then. Larvae concentrated also in group E, and the upper-middle part became the main breeding place. But this situation did not continue so long. On July 29, when density reached its peak, larvae were found abundantly in almost all groups, especially in group E, I and K, indicating that larvae began to concentrate in the lower part again. This tendency became more clear at the next census on Aug. 6, when larvae concentrated markedly in group I, J, and K in the lower part. Thereafter, density decreased rapidly and rice fields with moderate density level occurred sporadically in various groups.

The relation between the density levels of larvae and the stages of rice plant cultivation is shown in Table 4, for the period till July 7 during which various stages in rice plant cultivation coexisted on the same day. On May 8 to 19, moderate density level occured only in fallow fields, but this may be natural since the number of fallow fields itself was much larger than that of ploughed fields or nursery beds. In June when the numbers in respective stages did not differ extremely, the occurence rate of over-moderate (moderate, moderate-high, and high) levels was about 24, 15 and 23 % in fallow fields, ploughed fields and nursery beds, respectively. Moderate-high or high level did not occur in ploughed fields, so there may be some differences between ploughed fields and the others. But the differences, if any, seem not to be very significant. Therefore rice fields in various stages of rice plant cultivation will be collectively treated in the following.

Next, variableness in time of larval density level in the same rice field was examined by looking at the relation between the density levels at two successive censuses. The direction of change of density level in a rice field is thought to be greatly influenced by the trend of population density in the whole area, so the relation between density levels at successive two censuses is shown, separate-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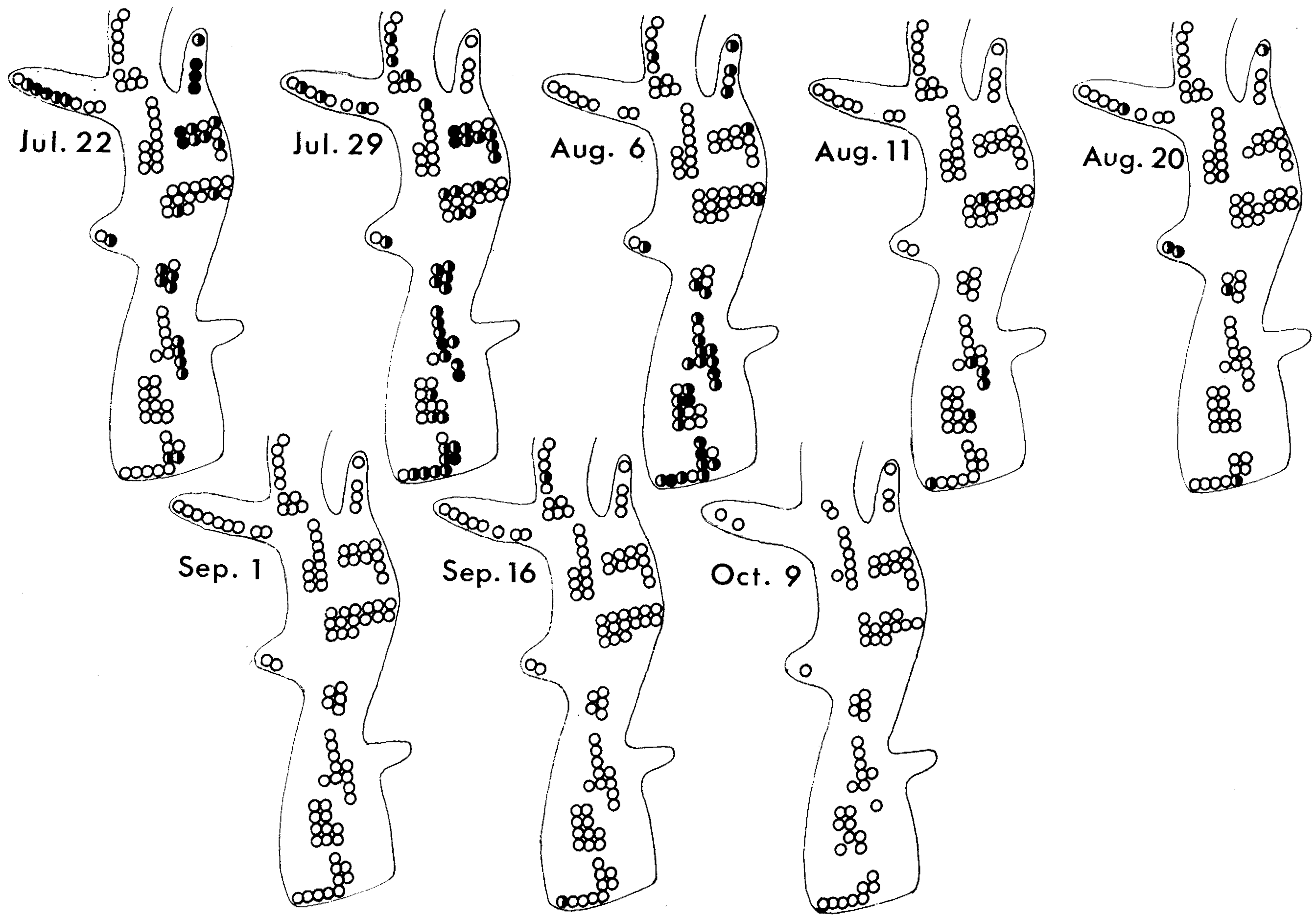

Fig. 2. Spatial distribution of rice fields with respective density levels of *Culex tritaeniorhynchus summorosus* larvae
○ : Low, ◑ : Moderate, ◕ : Modraete-high, ● : High. Rice fiedls with no water are not shown.

Table 4. Frequency distribution of density levels of *Culex tritaeniorhynchus summorosus* larvae in relation to the stage of rice plant cultivation.

No.	Date	Stage of rice plant cultivation	Density level			
			Low	Moderate	Moderate* -high	High
1	May 8	Fallow field	68	2		
		Ploughed field	5			
2	May 13	Fallow field	56	2		
		Ploughed field	11			
3	May 19	Fallow field	32	2		
		Ploughed field	17			
		Nursery bed	3			
4	May 25	Fallow field	45			
		Ploughed field	26	1		
		Nursery bed	13			
5	June 2	Fallow field	23	1		
		Ploughed field	31	5		
		Nursery bed	18	2		
6	June 9	Fallow field	11	5		1
		Ploughed field	20	6		
		Nursery bed	15	5		1
7	June 16	Fallow field	21	9	1	
		Ploughed field	33	4		
		Nursery bed	15	5	1	
8	July 7	Fallow field	14	2		
		Ploughed field	32	2		
		Nursery bed	12			
		Rice field	26	2		

* When density level was not determined by 3 dips between moderate and high, it was designated as moderate-high.

Table 5. Relation between density levels of *Culex tritaeniorhynchus summorosus* larvae at successive two censuses in the same rice fields, by population trend in the whole area. The number of rice fields falling into each category is presented.

Whole population level	At present census	At previous census				
		No water	Low	Moderate	Moderate -high	High
did not change *	No water	45	31	1		
	Low	11	106	2		
	Moderate		3	1		
	Moderate-high					
	High					
increased **	No water	64	29	5		
	Low	27	481	46	1	3
	Moderate	11	92	21	1	2
	Moderate-high		4	1		
	High		7	3		2
decreased ***	No water	40	34	4		
	Low	45	356	70	3	4
	Moderate	1	19	16	1	3
	Moderate-high			1	1	
	High			2		

* 2nd and 3rd censuses
** 5, 6, 7, 9, 10, 11, 14 and 16th censuses
*** 4, 8, 12, 13, 15 and 17th censuses

ly in 3 kinds of cases according to the trend of the whole density in the area, in Table 5. The figures below dotted lines show the numbers of rice fields where density levels became higher than those at previous census and the figures above the lines show the numbers of rice fields where density levels became lower. When the whole density was increasing, density levels became higher in many rice fields (118 in total), but became lower in considerable number of rice fields (58 in total), too. In 50 rice fields out of 58, the density changed to low level from over-moderate (moderate, moderate-high, and high) levels. When the whole density was decreasing, on the contrary, rice fields where density levels became lower were about 4 times as many as those where density levels became higher. But it is to be noted that the density levels in the 23 rice fields became higher in opposition to the direction of whole density. In 19 out of 23, density changed to over-moderate levels from low level. In both the cases mentioned above, the number of rice fields where density remained at over-moderate levels was much smaller compared with that where density level changed to low from over-moderate levels or *vice versa*. The former was 30 and the latter was 153, when the whole density was increasing, and 24 and 96, respectively, when it was decreasing.

When the density level in the whole area did not change, the number of rice fields where density increased was the same as that where density decreased. But it is noted that even in this case the number of rice fields where density level changed from low to over-moderate or *vice versa* was much more than that where density remained at over-moderate levels. The former was 5, while the latter was only one. Therefore it seems that density level in a rice field was changing from time to time, i.e., density changed from low to over-moderate level or *vice versa* in many rice fields only in a week or so.

This variableness of density level in a rice field is considered generally to be related to the variableness of the position of groups where rice fields with moderate or high level concentrated. But strictly speaking, two types of variableness were not throughly the same phenomenon, for the rice fields falling into over-moderate level were not necessarily the same between successive two censuses, even when the numbers of such rice fields in a group were the same. This situation becomes apparent by comparing, for example, group F on June 9 with June 16, and K on July 29 with Aug. 6.

So far, the density level for all larvae (1st, 2nd, 3rd and 4th instar larvae and pupae) was mentioned. But the density level of older larvae (4th instar larvae and pupae) is especially important for the ecology of this mosquito in relation to the epidemiology of Japanese encephalitis. Therefore the same sequential sampling table as used in the above was applied to the numbers of only older larvae in each rice field, to know the spatial distribution of rice fields where density of older larvae fell into moderate or high level. The result is shown in Fig. 3, together with the distribution of rice fields where density of all larvae fell into such levels, at least once, in the year. It is obvious

Table 6. Relation between number of times in a year at which densities for all larvae fall in over-moderate density levels and that for older larvae in each rice field. The number of rice fields falling into each category is presented.

No. times for all larvae	No. times for older larvae 0	1	2	3	4	Total
0	17					17
1	17	8				25
2	10	6	9			25
3	2	8	5	2		17
4	2	2	5	4		13
5					2	2
6		1				1
Total	48	25	19	6	2	100

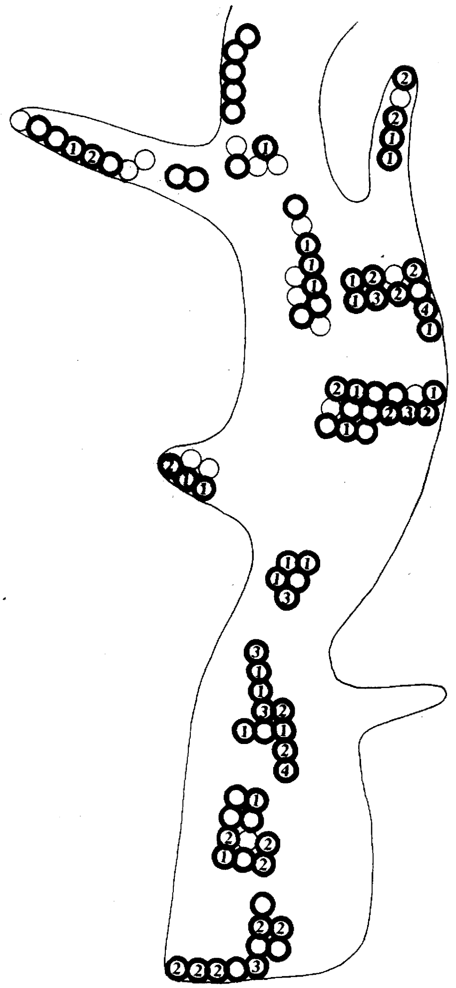

Fig. 3. The spatial distribution of older larvae of *Culex tritaeniorhynchus summorosus*. The figures in circles indicate the number of times when the density of older larvae fell into over-moderate (moderate, moderate-high or high) levels throughout the year in each rice field. Thick-lined-circles indicate that the density of all larvae fell into over-moderate levels, at least once, during the year.

from the figure that rice fields where density of older larvae fell into such levels were found in all groups from A to K. It means that adults emerged probably from all over the study area. Generally the over-moderate density levels of older larvae tended to occur in higher frequencies in the rice fields where the over-moderate density levels of all larvae occurred in high frequencies than in the rice fields where the frequencies were low, as is shown in Table 6. However, it is clear that in some rice fields where density levels for all larvae were over-moderate often, older larvae remained in low level throughout the year. Therefore it can be said that the frequencies of occurence of moderate or high density level for older larvae were different even in rice fields where the frequencies for all larvae were the same, though this tendency may have been emphasized more than the real situation owing to sampling error and sampling interval.

Discussion

The results of the present study showed clearly that larvae of the mosquito *Culex tritaeniorhynchus summorosus* tended to concentrate, at a certain time, in rice fields lying in some part of the whole rice field area in question (Fig. 2) and also that the position of such part changed gradually during the period of occurence (Fig. 2 & Table 5). Moreover the density level differed, sometimes markedly, among rice fields situated closely (Fig. 2).

The study was carried out during only one year in only one area and also some sampling errors may have been included. But it seems to be obvious that the above description represents one of the general features of spatial distribution of larvae of this mosquito. It agrees with many our experiences obtained in various areas since the onset of ecological studies of this mosquito in 1965. Variableness of the position of rice fields where density levels were relatively higher than in others, looks, at a glance, to be inconsistent with the observation by Nakamura *et al.* (1971) that rice fields with high density tended to be fixed. But it is necessary to be remembered that the study was done for only 2 months in the latter. Limited only in July and August as was in the latter, density levels were usually higher in some rice fields, for example, in H−2 and I−8 than in the others.

From the results it is apparent that the density level of larvae obtained from the census carried out in a relatively small number (10 or less) of rice fields will not, in many cases, be parallel with the density level in the whole area. Therefore it is recommended to increase the number of rice fields dipped, even though the number of dips taken from a rice field is decreased to only one, for estimating the relative density of the whole area in maximum efficiency (Wada *et al.*, 1971b). The small number of rice fields studied is probably one of the main reasons producing the discrepancy between seasonal prevalences of adults and larvae, observed sometimes in a same rice field area, as suggested by Buei *et al.* (1968). Perhaps adults collected in animal sheds or by dry ice traps must have included those which emerged from much wider area than that where population study of larvae was done.

Next, the processes leading to such concentrated distribution of larvae and shift of its centre during the year will be discussed. Pefore the transplantation of rice plant (in May and June), rate of water lodged surface area was smaller in the upper part than in the other parts. Many rice fields in the upper part had water only in small pits such as footprints, often covered by weeds. This may have been a main factor causing rare occurrence of moderate or high density level of larvae in this part, because *Culex tritaeniorhynchus summorosus* breeds preferably in ground pools with large surface area. But after transplantation (from July to September) there seems to have been no significant difference among conditions of rice fields lying in respective parts, so the processes leading to the concentrated distribution of larvae are thought to be more complicated.

Density of larvae in a rice field is determined both by the density of egg rafts laid and the survival rate of larvae in the rice field in question. Therefore the first requirement for the occurence of many larvae is that many egg rafts have been laid in that rice field. Where egg rafts are laid is, in turn, affected by many factors (see e.g., Clements, 1963). Distribution of resting places for engorged or gravid females may play a role in determining the egg laying place, because they determine the place from which flights leading to oviposition start. However, the resting place does not seem very important in the present case, since the usual flight range of this mosquito seems to be at least 1.0km (Wada *et al.*, 1969), so the dispersal within the study area is probably unrestricted. Orientation of flying mosquitoes is affected by topographical features, vegetation, wind direction and its velocity and other meteorological factors etc. Furthermore the physical and chemical natures of water may play an important role. In *Culex tritaeniorhynchus summorosus*, it is quite unknown how those factors are operating actually in deciding the oviposition place, however, some speculations are possible.

As the position of the part including rice fields with many larvae changed markedly even in a week (see distribution maps on July 22 and 29th of Fig. 2), so factors leading to such changes must also have had the nature changeable in a week. In this point, topography and vegetation may be excluded, though they are important just as underlying conditions. (The flying route of *Anopheles gambiae melas* seeking hosts is said to be greatly affected by vegetational features (Giglioli, 1965). Also in *Culex tritaeniorhynchus summorosus* it is probable that the relative importance of topographical and vegetational features may increase in the case of little wind.) Physical and chemical natures of water may be also excluded, because they seem to have been different not among the parts of whole area, but among individual rice fields. Therefore it follows that the direction and velocity of prevailing wind may be the most important factor causing the inclination and shift of egg laying part within a considerably large area. Predominating effect of wind on orientation of flying mosquitoes is consistent with various observations in laboratory and in field, too (e.g., Kennedy, 1940 and Klassen & Hocking, 1965).

It is highly probable that physical and chemical natures of water act in a short distance from water and play an important role in the final descent on water surface. The difference of density levels of larvae observed sometimes among rice fields situated closely in the same group may be, at least partly, explained by the difference in natures of water.

The second requirement for the occurrence of many larvae in a particular rice field is that survival rate during larval stages is relatively higher in that rice field. Survival rate of larvae is also affected by many factors such as temporary drainage (Ogata & Nakayama, 1963; Makiya, 1970), water flow (our unpublished data), insecticides applied to pests of rice plant (Ori *et al.*, 1963; Ogata & Nakayama, 1963), food supply (Nakamura *et al.*, 1971), natural enemies (our unpublished data), duck weed coverage on water surface (Makiya, 1970) and so on. In the present study, the intensities of such factors in each rice field were not fully recorded, however it was observed that some of those factors operated differently among rice fields. Also the fact that the rates of occurrence of older larvae were low in some rice fields (Fig. 3 & Table 6) may be an indication that the intensities of such factors affecting the survival rate differ among rice fields. This seems to contribute, at least partly, the difference of density level among rice fields situated closely in the same group.

Summarizing, gravid females may be concentrated in some part mainly by the direction and velocity of wind, then attracted to some rice fields by physical and chemical natures of water, and many adults may emerge from some of those rice fields where the survival rates of larvae are relatively high. Difference of both the density level and the pattern of seasonal prevalence of larvae observed among rice fields in the same area is probably produced through these three steps. However these ideas are only a hypothesis at present and require many further investigations. Then the comparison of distribution pattern of *Culex tritaeniorhynchus summorosus* with that of *Anopheles sinensis*, another dominant species breeding in rice fields, will probably give a useful information to examine the hypothesis.

Although it depends on future study to examine the propriety of the hypothesis presented, it can be said certainly even now that one rice field should be considered as one of natural units for the larval population in a rice field area, because factors affecting the larval survival rate differ, frequently markedly, among rice fields situated closely and moreover larval movement among rice fields is probably difficult at usual circumstances. This aspect seems to be very significant for understanding the processes of population fluctuation of this mosquito, for density effects by overcrowding of larvae (though the effects may be expressed in the adult stage) may act in some rice fields, while density in many other rice fields is remained low.

It is reasonable to expect that some sorts of density effects play a significant role in fluctuation of *Culex tritaeniorhynchus summorosus* population, as in populations of many other insects. So, to make clear the mode of density effects in nature must be undoubtedly one of the most important points for understanding the processes of population fluctuation of this mosquito.

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Spatial distribution of larvae of the mosquito *Culex tritaeniorhynchus summorosus* in a rice field area

水田地帯におけるコガタアカイエカ幼虫の空間分布

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摘　　　　要

長崎県に多い山間の水田地帯で，コガタアカイエカ幼虫の空間分布を調査した．調査地にある約15ヘクタール（およそ 500枚）の水田のいろいろの部分から全部で 100枚の水田を選び， 5月から 10月まで，原則として週に1回調査をした．その結果，コガタアカイエカ幼虫の密度の高い水田は，ある調査日には特定の場所に集中している傾向があること，その場所の位置は調査日ごとに変化していくこと，更に，隣合った水田で幼虫密度が著しくちがうことも珍しくないことが明らかになった．この結果は，広い地域のコガタアカイエカ幼虫の密度を知るためには，その地域内のいろいろの場所でなるべく多くの水田を調査しなければならないこと，及び，コガタアカイエカ幼虫個体群にとっては，個々の水田が1つの小単位となっていることを示している．後者は，コガタアカイエカの個体数変動の過程を解明していく上で極めて大切な観点であると思われる．何故ならば，大部分の水田の幼虫密度が低い時でも，一部の水田では過密による密度効果が働く可能性があるからである．